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Importance of Genetic Diagnostics in Adult-Onset Focal Segmental Glomerulosclerosis

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Abstract
Focal segmental glomerulosclerosis (FSGS) is a histological pattern of podocyte and glomerulus injury. FSGS can be primary and secondary to other diseases or due to a genetic cause. Strikingly, genetic causes for adult-onset FSGS are often overlooked, likely because identifying patients with genetic forms of FSGS based on clinical presentation and histopathology is difficult. Yet diagnosing genetic FSGS does not only have implications for prognostication and therapy but also for family and family planning. In this case series, we present 3 adult patients who presented with advanced renal disease with the histological picture of FSGS and proved to have a genetic cause of the disease, namely, variants in *INF2*, *COL4A4* and *HNF1B*, respectively. We show the possibilities of identifying genetic FSGS based on clinical clues of a positive family history, early age at onset of disease, and/or severe therapy-resistant disease. We discuss ways to select the method of genetic testing for individual patients. Finally, we examine how the judicious use of genetic investigations can obviate potential harmful diagnostic procedures and direct clinical decisions in patients and their relatives.

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Background

With the advances in genetic testing methods, genetic analysis is an increasingly important diagnostic tool in nephrology [1]. This is also the case for genetic focal segmental glomerulosclerosis (FSGS), which is the focus of this paper.

FSGS is a histological pattern of podocyte loss and glomerular injury. It is characterized in a renal biopsy, by segmental sclerotic lesions in at least one glomerulus (observed with light microscopy) and effacement of the podocyte foot processes (observed with electron microscopy [EM]) [2, 3]. The underlying causes for FSGS are heterogeneous [4, 5].

FSGS is traditionally categorized according to those underlying causes, namely, primary (often involves a circulating factor causing podocyte dysfunction) and secondary to a nonrenal disease and genetic FSGS [4, 6]. Depending on the underlying cause, the patients can present with proteinuria, or nephrotic syndrome (most in primary FSGS), and end-stage renal disease (ESRD), or progress to ESRD over the course of 5–10 years [7].

There are no clear-cut clinical or histopathological findings to distinguish genetic FSGS from other types [8]. However, there are several hallmarks of genetic disease. Namely a positive family history, early age at onset of disease (~30% of FSGS with an onset before 25 years of age is genetic), and uncharacteristically severe and/or steroid-resistant disease [8–11]. Conversely, because genetic disease often presents at a young age, it is often unjustly overlooked in adult-onset FSGS patients [11].

With the advances of genetic testing, however, diagnosing genetic FSGS has become much more feasible over the past few years. Not only because over 50 genes are currently known to be involved in FSGS, but also since the costs and turn-around time for genetic tests are continuously dropping, increasing their availability in daily clinical practice [8, 11–15].

The technique most frequently used for genetic testing is next-generation sequencing (NGS) [8, 11–14]. NGS can identify disease-causing mutations in the entire genome (whole-genome sequencing), the protein-coding regions (whole-exome sequencing), or a specific set of genes of interest (targeted gene panel [TGP]) [16]. For instance, the TGP on FSGS in online supplemental Table 1 (for all online suppl. material, see www.karger.com/doi/10.1159/000499937) contains the classic FSGS genes *NPHS1* and *NPHS2* as well as genes recently associated with FSGS such as the *COL4A3–5* genes (the Alport syndrome genes) and *PAX2* (involved in nephrogenesis). Selecting the right NGS test is essential, to be able to come to a diagnosis with limited risk of the incidental findings that testing many genes (e.g., whole-exome sequencing) can bring.

Despite the abovementioned challenges, considering a genetic cause in adult-onset FSGS patients is important as it can have a large impact on the patient and his/her family members. Here, we present 3 patients with adult-onset chronic kidney disease who were clinically and histopathologically diagnosed with FSGS and were shown to carry a genetic cause thanks to a close collaboration between nephrologists, pathologists, and clinical geneticists. We use these cases to discuss the expanding possibilities of diagnosing genetic FSGS and the clinical implications of such a diagnosis.

Case 1: FSGS with ESRD at a Young Age

A 30-year-old man with asymptomatic 2 g/day proteinuria at age 20 and ESRD at age 29 (no signs of nephrotic syndrome, Table 1) was referred to our nephrogenetics out-patient clinic. There was no family history of renal disease. Renal biopsy at age 29, when the patient developed ESRD, showed FSGS (Fig. 1a), with 80% globally sclerosed glomeruli and partial podocyte foot process effacement (Fig. 1d) [17]. The patient was referred because he was planned to undergo a kidney transplant from a family member.

Due to the young age of onset of proteinuria in this patient, there was a marked probability of genetic FSGS, and a diagnostic TGP analysis for FSGS was performed (online suppl. Methods 1 and Table 1). This revealed a heterozygous known pathogenic mutation in the *INF2* gene (OMIM610982, Table 2) [18–21]. The mutation had been previously detected in FSGS patients, though one should note that no functional assessment of that specific mutation was performed [18]. Mutations in *INF2* are known to be a major cause for autosomal dominant FSGS [22–24].

To adequately counsel family members, segregation analysis was performed in the patient's healthy parents. The father did not carry the mutation and later successfully donated a kidney to our patient. In the otherwise healthy mother, a 20% mosaicism for the *INF2* mutation was detected in DNA from peripheral blood. The mother was referred for extensive health screening, which revealed no abnormalities. Since she had had a son with *INF2* mutation, it must therefore be present in the germline and thus possibly have been passed down to the patient's siblings. One sibling decided on testing (revealing no *INF2* mutation) and one decided to undergo periodic evaluation of renal function. The patient's young child will be counseled regarding presymptomatic genetic testing when it is of age. As the earliest presentation reported in literature is at 7 years of age, the child will undergo proteinuria screening [25].

Next to the implications for family members, the molecular diagnosis impacted the patient's care directly. Mutations in *INF2* can also be associated with dominant intermediate Charcot-Marie-Tooth disease, thus the patient was neurologically evaluated, showing no abnormalities [26]. Additionally, the patient and his

Table 1. Age at first presentation, laboratory findings, and morphological findings per case

Case number	Age at first presentation, years	Positive family history	Clinical diagnosis	eGFR at presentation (CKD-EPI) [47], mL/min/1.73m^2	Laboratory analysis at presentation	Renal ultrasound results	Light microscopy	Immunofluorescence microscopy	Electron microscopy	Histological classification [17]
Case 1 UMCU_ NG_ 012_01	20	No	Secondary FSGS	<20	*Blood* Albumin normal Lipids normal PT and APTT normal *Urine* Protein (2 g/day)	Echodense kidneys, otherwise no abnormalities. Length 9.9 and 9.8 cm (normal). Changes likely due to CKD	FSGS with 80% glomerulo-sclerosis	No immunoreactivity	Partial podocyte effacement	FSGS NOS
Case 2 UMCU_ NG_ 044_01	50	Yes	Secondary FSGS	90	*Blood* Albumin normal Triglycerides high PT and APTT normal *Urine* Protein (1.6 g/day) 30 erythrocytes/μL	No abnormalities. Length 12.5 and 11.6 cm (normal)	FSGS with 50% glomerulo-sclerosis	A specific immunoreactivity for IgA and IgM	Partial podocyte effacement Thin basement membrane (mean 172 nm)	FSGS NOS
Case 3 UMCU_ NG_ 100_01	33	Yes	FSGS, etiology unknown	39	*Blood* Albumin normal Triglycerides high PT and APTT normal *Urine* Protein (0.6 g/day)	No abnormalities. Length 10.2 and 10.5 cm (normal)	FSGS with 45% glomerulo-sclerosis	No immunoreactivity	No material	FSGS NOS

APTT, activated partial thromboplastin time; CKD, chronic kidney disease; eGFR, electronic glomerular filtration rate; FSGS, focal segmental glomerulosclerosis; Ig, immunoglobulin; NOS, not otherwise specified; PT, prothrombin time; SRNS, steroid-resistant nephrotic syndrome.

partner wanted to have more children. After counseling, they opted to try to conceive via preimplantation genetic diagnostics, an in vitro fertilization procedure where an embryo *without* the *INF2* mutation is transferred into the uterus [27]. At time of this publication, this has not yet led to an ongoing pregnancy.

Case 2: FSGS with a Family History of ESRD

A 50-year-old obese woman (BMI 34) of Hindustani Surinam descent (Table 1) presented in the referring hospital with mild chronic kidney disease (eGFR = 90), distinct proteinuria (1.6 g/day, no signs of nephrotic syndrome), and erythrocyturia of 30 cells/μL. Her parents had ESRD, both with an age of onset around 60 years, of which the father was diagnosed as having diabetic nephropathy. In our patient, renal biopsy displayed FSGS (Fig. 1b), with 50% globally sclerosed glomeruli, thought to be secondary to a metabolic syndrome. However, because of the erythrocyturia, the referring nephrologist wondered if *COL4A3–5*-related disease (mutations in these genes are detected in patients with thin basement membrane nephropathy and classical Alport syndrome) might play a role in this patient's phenotype.

To assess this possibility, the renal biopsy was revised with EM. This showed a thin GBM with a mean thickness of 172 nm, (Fig. 1e), which was well below the lower limit of 252 nm determined in our center for normal GBM thickness for females and also below the lower limit of 215 nm for the normal thickness for females reported in literature, further pointing toward *COL4A3–5*-related disease [28, 29]. Therefore, the diagnostic TGP analysis on FSGS was performed (online suppl. Methods and Table 1). This analysis includes the *COL4A* genes, since mutations in these genes have been shown to cause a histological FSGS phenotype in some cases [28, 30–34]. The TGP analysis showed a heterozygous likely pathogenic mutation in the *COL4A4* gene (OMIM120131, Table 2), with no variants in other FSGS-linked genes [19–21].

COL4A4 codes for the type IV collagen alpha-4 chain, a protein essential to the GBM [35]. Heterozygous mutations in *COL4A4* have been associated with familial hematuria [36]. There are reports suggesting that specific mutations in *COL4A4* or unknown

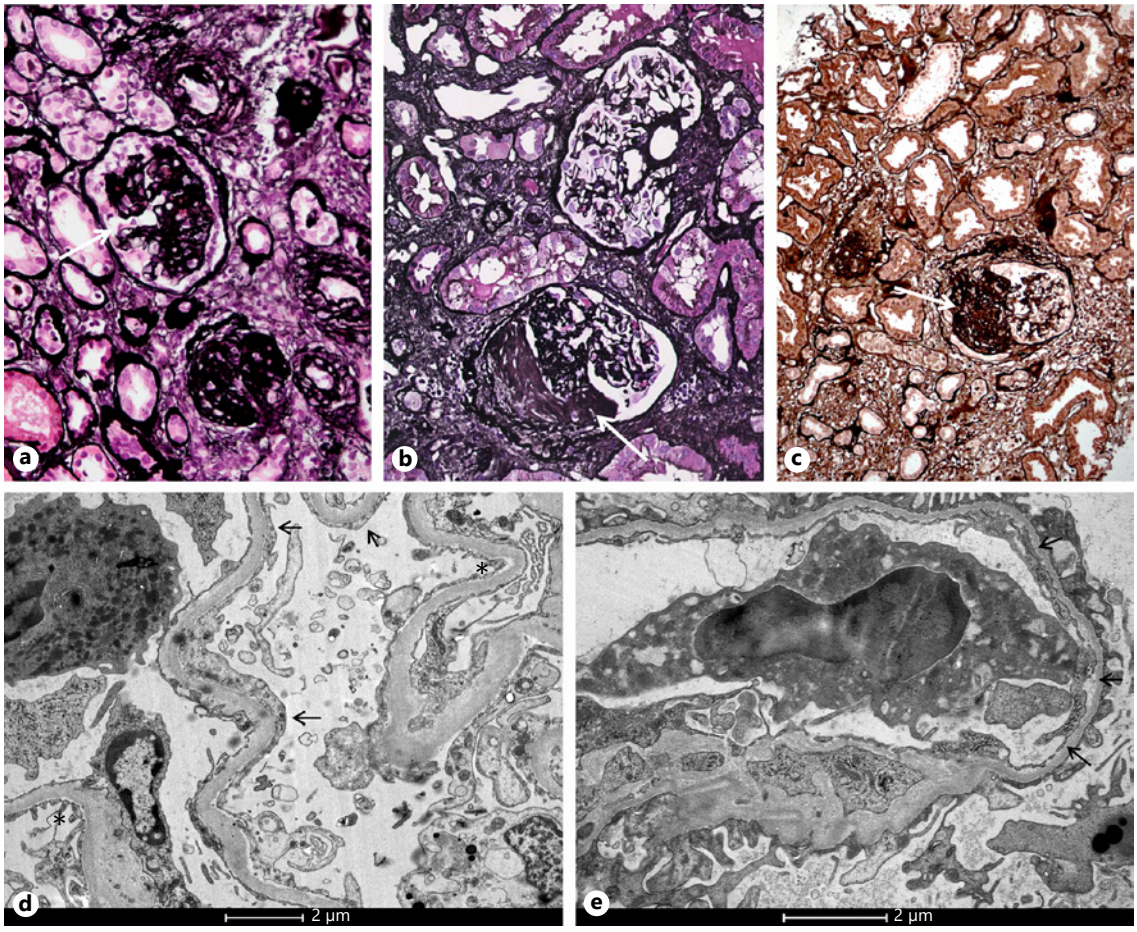

Fig. 1. Kidney biopsy images in the 3 cases. Light microscopy (Jones staining) showed glomeruli with segmental sclerosis (arrows) in case 1 (**a**), case 2 (**b**), and case 3 (**c**). Electron microscopy of case 1 showed partial foot process effacement, with areas of intact foot processes (*) alternating with areas with foot process effacement (arrows, **d**). In addition to partial foot process effacement, EM of case 2 also showed a thin GBM thickness with a mean of 252 nm (arrows, **e**).

genetic modifiers might cause FSGS lesions in heterozygous carriers, while others suggest that heterozygous *COL4A3–5* mutations are the most frequent underlying cause in patients with FSGS on biopsy [37–40]. It is clear that the penetrance of renal disease in carriers of heterozygous *COL4A3–4* mutations is far from complete [37–40]. There is debate over whether this is best called autosomal dominant Alport syndrome, or for example, *COL4A3–4*-related disease [37–40].

The specific mutation detected in our patient has not been described as pathogenic before. However, the variant causes the substitution of a highly conserved glycine residue in the collagen triple-helix repeat by a more bulky amino acid (Table 2). Based on the fact that most known pathogenic mutations in *COL4A4* lead to similar substitutions, the mutation was classified as "likely pathogenic." Segregation analysis was performed, and the mother (no diabetes) proved to be a carrier for the same mutation. The presence of the *COL4A4* variant in 2 affected family members, along with erythrocyturia and a thin GBM, likely explains at least a part of our patient's *COL4A3–5*-related disease phenotype. With this, it is important to note that people of Hindustani Surinam descent are known to have higher risk of metabolic syndrome, which likely also played a role in this family's renal phenotype(s) [37, 41].

Genetic counseling was offered to the patient's children. Furthermore, the finding of a *COL4A4* likely pathogenic variant triggered the referring nephrologist to prescribe Lisinopril, as the patient needed antihypertensive medication and ACE-inhibition is also used to attenuate renal function decline in Alport syndrome [42].

Case 3: "IgA-Related FSGS" with a Family History of ESRD

An otherwise healthy 33-year-old man presented with an eGFR of 39 and proteinuria (0.6 g/day, no signs of nephrotic syndrome). The family history revealed that the mother had died with ESRD

Table 2. Molecular diagnosis, including the performed genetic testing and information on the genetic variant, per case

Case number	Genetic testing performed	HGNC-approved gene name (transcript number)	OMIM number	Variant	Homozygous or heterozygous	Variant type	Reference/in silico predictions [18–21]
Case 1 UMCU_ NG_ 012_01	FSGS	*INF2* (NM_022489.3)	610982	c.217G>A p.(Gly73Ser)	Heterozygous	Pathogenic	Barua et al. [18] (no functional analysis of this variant) PolyPhen HumDiv score 1.000, sensitivity 0.00, specificity 1.00 Polyphen HumVar score 1.000, sensitivity 0.00, specificity 1.00 SIFT score 0.13 (tolerated) Not present in the gnomAD database
Case 2 UMCU_ NG_ 044_01	FSGS	*COL4A4* (NM_000092.4)	12131	c.2038G>C p.(Gly680Arg)	Heterozygous	Likely pathogenic	PolyPhen HumDiv score 1.000, sensitivity 0.00, specificity 1.00 Polyphen HumVar score 1.000, sensitivity 0.00, specificity 1.00 SIFT score 0.00 (deleterious) Not present in the gnomAD database
Case 3 UMCU_ NG_ 100_01	FSGS PAX2 Sanger sequencing Full diagnostic renal diseases ('RENome')	*HNF1B* (NM_000458.3)	189907	c.908G>A p.(Arg303His)	Heterozygous	VUS	PolyPhen HumDiv score 0.998, sensitivity 0.27, specificity 0.99 PolyPhen HumVar score 0.877, sensitivity 0.71, specificity 0.89 SIFT score 0.04 (deleterious) Not present in the gnomAD database

Arg, arginine; del, deletion; FSGS, focal segmental glomerulosclerosis; Glu, glutamic acid; Gly, glycine; HGNC, HUGO gene nomenclature committee; His, histidine; OMIM, online Mendelian inheritance in man®; Ser, serine; VUS, variant of unknown significance.

at age 50, most likely due to hypodysplastic kidneys. Renal ultrasound in the patient showed no abnormalities and normal sized kidneys (Table 1). In the referring hospital, renal biopsy was classified as FSGS secondary to IgA depositions. The patient wondered if he could pass on the disease to his children.

Biopsy revision at our facility showed FSGS (Fig. 1c) with 45% of glomeruli globally sclerosed, but no immunoreactivity for IgA. There was not enough material to perform EM. Since the diagnosis of IgA nephropathy was doubtful, genetic diagnostics using the FSGS TGP analysis was performed (online suppl. Methods 1 and Table 1). This did not lead to a molecular diagnosis. Due to the high clinical suspicion, the analysis was expanded to a larger panel of ~225 published renal genes. This revealed a heterozygous variant of unknown significance in the *HNF1B* gene (OMIM189907, Table 2) [19–21, 43].

The variant had not been observed before in patients or large healthy control populations, in silico predictions suggest a possible pathogenic effect (Table 2), and the variant segregated in the patient's deceased parent. Laboratory work-up in our patient for glucose, electrolyte, and liver enzyme imbalances associated with *HNF1B*-related disease showed no clear abnormalities; however, genotype-phenotype correlations can be unclear [44, 45]. The *HNF1B* variant might thus be causal in our patient's disease and the mother's renal hypodysplasia. This is underscored by studies showing that HNF1B works as a modifier on PAX2, in which gene mutations are known to cause both isolated congenital anomalies of the kidney and urinary tract (CAKUT, such as hypodysplasia) as well as FSGS [46–48]. Also, mutations in *HNF1B* sometimes cause a CAKUT phenotype without abnormalities in other organs [46, 47]. Hence, it could be that mutations in *HNF1B* also lead to FSGS. Publication of this, to our knowledge first ever, case will hopefully stimulate further research into the *HNF1B*-FSGS relationship.

Though the patient cannot be conclusively diagnosed, the combination of the variant and the positive family history has led to all at-risk family members receiving advice for periodic evaluation of renal function.

Discussion

The cases presented in this paper show that although the identification of a genetic cause for FSGS presenting at an adult age can be complex, an adequate diagnosis can have far-reaching implications. That the cases were rediagnosed as genetic FSGS is due to the multidisciplinary approach with input from a nephrologist, pathologist, and clinical geneticist. These specialists discussed the possibility of genetic disease and the appropriate application of genetic testing for each patient individually. We discuss the items at the core of this discussion in detail below.

First, it is vital to recognize that though patient characteristics can give clues on patients with high risk of a genetic disease, not all patients display those hallmarks of genetic disease [8, 9, 11]. Similar to the *INF2* case we presented, a family history might be absent due to germline mosaicism, or mutations that are recessive, de

novo or incompletely penetrant [14]. Additionally, though a young age at presentation is an indication of genetic disease, our *COL4A4* patient presented at 50 years of age [9]. The notion that genetic renal disease can present later in life is underscored by our recent finding that the classic pediatric disease nephronophthisis actually can present with ESRD to up to 61 years [49].

Second, one should consider the appropriate NGS scale for each patient. In order to test a sufficient number of genes without risk of incidental findings, we apply a tiered approach, starting with the analysis of TGPs that are limited to strictly FSGS-associated genes. If a limited TGP does not yield a diagnosis, one can opt to analyze a larger panel (as we did for our *HNF1B* case), or to perform whole-exome sequencing to look for variants in genes not yet associated with the patient's phenotype. To make such a step-up process even easier, we decided in 2017 to derive all TGP analyses from whole-exome sequencing data. Adequate pre- and posttest counseling (described by our group previously [38]) regarding analyses of the whole-exome data should be offered to patients, as these can reveal incidental findings.

With the continuous decrease in cost and turn-around time of NGS, the precise selection of patients and a step-up NGS method will likely become less of a question [15]. However, genetic testing should always be applied after consideration of the prognostic and therapeutic implications of finding a genetic variant for the patient and his/her family members.

For the patient, it can provide information on useful treatment strategies. Though genetic FSGS generally does not respond to corticosteroid treatment, other drugs might be beneficial, such as ACE-inhibition in *COL4A*-related disease [42, 50, 51]. Furthermore, a molecular diagnosis is relevant when deliberating on a renal transplantation. First, because it usually offers a favorable prognosis with respect to recurrence in a renal graft, since chances of this are very low in genetic FSGS [52]. Second, if living-related transplantation is considered, it is safest to have a genetically unaffected family member donate [53]. For this reason, we tested the *INF2* patient's parent before proceeding to donation.

Family members are impacted, as they are at risk of also developing FSGS. Those at risk should be offered counseling on genetic testing and/or (presymptomatic) evaluation of renal function [53]. Likewise, *future* children of a genetic FSGS patient could inherit the disease. It is our experience that the knowledge that the disease is genetic is very important for patients when contemplating how to establish their family. As we saw in our *INF2* case, the options for not passing the disease on not only include having less or no children but also advanced techniques such as preimplantation genetic diagnostic, when locally available [54].

In conclusion, the cases presented in this paper show that a genetic diagnosis in adult-onset FSGS can have far-reaching consequences not only for the patient but also for his/her family (planning). Identification of patients with a higher likelihood of a genetic FSGS often proves challenging, though there are several hallmarks of genetic disease. Currently, we apply a tiered method to genetic testing, to limit incidental findings. In the future, a genetic-first approach could obviate invasive renal biopsies [55]. The probability of a monogenic disease and the potential impact of a genetic diagnosis should be considered in the diagnostic work-up of all adult-onset FSGS cases.

Statement of Ethics

The patients described in this paper have given their informed written consent for their anonymized data to be included in this study. In the Netherlands, there is no need for Institutional Review Board permission to publish anonymized, retrospective patient data; therefore, no such permission was sought.

Disclosure Statement

The authors declare no conflicts of interest, financial or otherwise.

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